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Association of Serum α1 Acid Glycoprotein with Low Grade Chronic Inflammation in Pathogenesis of Diabetes Mellitus.

Shamim Shaikh Mohiuddin*

Assistant Professor, Department of Biochemistry, College of Medicine. Imam Abdulrahman Bin Faisal University, PO box- 1982, Dammam-31441, Kingdom of Saudi Arabia

***Corresponding Author:** Dr. Shamim Shaikh Mohiuddin, Assistant Professor, Department of Biochemistry, College of Medicine. Imam Abdulrahman Bin Faisal University, PO box- 1982, Dammam- 31441, Kingdom of Saudi Arabia.

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Abstract

Pathogenesis of type 2 as well as type1diabetes mellitus is observed to be closely associated with acute phase response which is predominately cytokine-mediated. In this present study I try to test this hypothesis by estimating circulating α1 acid glycoprotein in freshly diagnosed type 1 (T-1), freshly diagnosed type 2(T-2) as well as type 2 diabetic patients under oral hypoglycemic drugs for duration of at least five years. α1 acid glycoprotein is considered as a prominent member of acute phase protein and very important tools for diagnosis for low grade chronic inflammatory reaction. Thirty normal controls to match the age and sex of the test groups were also studied. The level of this parameter was also correlated with their random plasma glucose values and BMI.

The valueof α1 acid glycoproteinis significantly elevated in the T-2 patients (p <.00001) in comparison with the controls. In case of T-1 patients the level of α1 acid glycoprotein was not found statistically significant (p value 0.275). Interestingly in either of the types, no correlation was found with the degree of hyperglycemia or BMI.

By the above results and findings is can be definitely postulated that a low grade inflammatory process is surely associated in the pathogenesis of type 2 diabetes. But for type 1 diabetic patient the result is contradictory. This can be further explored for further diagnosis, management and follow up.

Keywords: *α1 acid glycoprotein; Acute phase proteins; Acute phase reactants; Low grade chronic inflammation; Diabetes mellitus*

Abbreviations: CRP: C-reactive Proteins; IL-1: Interleukin-1; IL-6: Interleukin-6; TNF: Tumor Necrosis Factor; RBS: Random Blood Sugar; LDL: Low Density Lipoprotein

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Introduction

Diabetes Mellitus is a syndrome with disordered metabolism and inappropriate hyperglycemia due to either deficiency of insulin secretion or to a combination of insulin resistance and inadequate insulin secretion to compensate [1]. Diabetes Mellitus is one of the

most common major public health problems having worldwide distribution. It is not age selective and affects all sections of population posing a major health challenge of the 21st century. World health Organization (WHO) has declared it to be a global disease of epidemic proportions. It is estimated that by 2025, an estimated 300 million people will have diabetes, most of whom will inhabit of China, India and United States [2]. Although hyperglycemia is the main characteristic of all form of diabetes mellitus, the pathogenic mechanism by which hyperglycemia arises differs widely. Some forms of Diabetes mellitus are characterized by an absolute insulin deficiency or a genetic defect leading to defective insulin secretion; where as other forms share insulin resistance as their underlying etiology [3].

This recent explosion of interest in the notion that chronic low grade inflammation and activation of the innate immune system are closely involved in the pathogenesis of type 2 diabetes mellitus was first proposed in 1997-98 [4]. During acute inflammatory states or secondary to certain type of tissue damage, the levels of certain proteins in plasma increased. These proteins are called acute phase protein or acute phase reactants and include C-reactive protein, (CRP), α-1 antitrypsin, ceruloplasmin, α-1 acid glycoprotein, haptoglobin and fibrinogen. The elevation of the levels of these proteins varies from as little as 50% to as much as 1000 fold as in case of CRP. In acute inflammatory state such as surgery, myocardial infarction, infection and tumor; concentration of these protein rise significantly [5]. These proteins are believed to play a role in body's response to inflammation. For example, C-reactive protein can stimulate the classical complement pathway; α-1 antitrypsin can neutralize certain proteases released during acute inflammatory state. Interleukin-1 (IL-1), a polypeptide released from mononuclear phagocytic cells, is the principle but not the sole stimulator of the synthesis of the majority of acute phase reactants by hepatocytes. Additional molecules such as interleukin-6 (IL-6) are involved and they as well as IL-1 appear to work at the level of gene transcription [6].

Several studies after that have shown that circulating markers of inflammation, acute phase reactants or interleukin-6 (IL-6) are strong predictors of the development of type 2 diabetes [7,8]. T-2 ((Type 2) diabetes mellitus is seen to be related with increased blood concentrations of markers of the acute-phase response known as α1 acid glycoprotein and also interleukin-6, which is the main cytokine mediator of the response. The dyslipidemia which is very much commonly seen in Type 2 diabetes (hypertriglyceridemia and low serum levels of HDL cholesterol) is also a feature of natural and experimental acute-phase reactions. We review evidence that a long-term cytokine-mediated acute-phase reaction occurs in Type 2 diabetes and is part of a wide-ranging innate immune response [9]. The role of acute phase reactants in the development of type 1 diabetes mellitus is not very clear. α1- acid glycoprotein is one of the important acute phase reactants. The level of this inflammatory markers in the pathogenesis of type 1 and type 2 Diabetes mellitus was of interest.

Schmidt's excellent review [10] covers historical aspects of work that dates back the 1940's when the seromucoid proteins were first described. The major portion of plasma seromucoids has since been recognized as α1- acid glycoprotein. Among the plasma proteins α1- acid glycoprotein is unique because of its low pI which is 2.7-3.5. Its molecular weight is ~40000. It contains 45% carbohydrate as hexose, hexamine and sialic acid in equal proportion. The antigenic determinant (epitope) utilized in immunochemical assays, however, is on the polypeptide moiety. It has an excellent solubility in water and polar solvents and has a high negative charge density at pH 7.4. Treatment with neuraminidase followed by isoelectric focusing reveals polymorphism in the α1 region. Though polymorphism has no known clinical significance but because of its α1- acid glycoprotein migration on agarose or cellulose electrophoresis may differ slightly from one specimen to another. α1- acid glycoprotein is associated with inflammation. Following an acute inflammatory episode increased levels of α1- acid glycoprotein observed, i.e. it is an acute phase reactant. A certain degree of homology exists among the amino acid sequence of α1- acid glycoprotein, the immunoglobins and haptoglobin α chain which suggest a common ancestry or role of the immune system [6]. Early work indicated that the liver was the only site of synthesis of α1- acid glycoprotein. More recent studies have shown that under certain well defined condition, some tumors are able to synthesize α1- acid glycoprotein. Catabolism of α1-acid glycoprotein proceeds first by desialation followed by rapid degradation in liver within minutes [6]. The clinical value of α1- acid glycoprotein determination is currently limited to monitoring the acute phase reaction. However increased serum occurs in rheumatoid

arthritis, systemic lupus erythematosus, Crohn's disease, and malignant neoplasms, especially those with metastasis and large tumor masses and myocardial infarction. Decreased level occurs in malnutrition, severe hepatic damage and severe protein loosing gastro-enteropathy [6]. The acute-phase proteins are mainly synthesized in the liver, stimulated by cytokines, mainly interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF) which are produced in macrophages, monocytes, endothelium and many other cells in the body [11]. The acute-phase proteins have many activities that in general contribute to host defense, healing and adaptation to insult. For example, proteinase inhibitors such asa1-antitrypsin control proteinases re-leased by phagocytes, fibrinogen has a major role in haemostasis, and ceruloplasmin, haptoglobin and metallothionene are antioxidants protecting against toxic oxygen metabolites produced at the site of injury and inflammation [12].

Materials and Method

Aims and objective

1. To detect the elevation of α1 acid glycoprotein, if any, in newly diagnosed untreated type 1 diabetes mellitus patients, in newly diagnosed untreated type 2 diabetes mellitus patients and in patient of type 2 diabetes mellitus under treatment for at least five years.
2. Compare the levels in newly diagnosed untreated type 2 diabetes mellitus patient with type 2 diabetes mellitus patients under treatment for at least 5 years.
3. Compare the level of this inflammatory marker of newly diagnosed untreated type 1 diabetes mellitus with newly diagnosed untreated type 2 diabetes mellitus patients.

Participants

Subjects were selected from various clinics and hospitals in Mangalore, India. Height and weight of all subjects were recorded and body mass index was calculated. None of the ninety two volunteers were alcoholics or smokers. The participants did not suffer from chronic inflammatory diseases like asthma, chronic bronchitis, and rheumatoid arthritis as was ascertained by clinical history. The study was approved by institutional ethical committee of Kasturba Medical College, Mangalore, India.

Materials

5 ml blood was collected in plain bottle. Informed consent was taken from the individual subjects prior to blood collection. Blood was taken from antecubital vein of the subjects and α-1 acid glycoprotein assay in serum was carried out by the method of Winzler RJ., *et al*. [13].

Principle of the test

After removing heat coaguable proteins with perchloric acid, the orosomucoid which remains in the solution is precipitated by phosphotungstic acid and estimated by determining it carbohydrate content by reaction with an orcinol-sulphuric acid reagent, or its nitrogen by Kjeldahl nesslerization or its tyrosine content using Folin Ciocalteau reagent. The last of these is employed in the technique given below.

Procedure

1. 0.5 ml serum was added to 4.5 ml sodium chloride solution followed by 2.5 ml of 1.8 mol/L perchloric acid drop wise with shaking.
2. After 10 minutes it was filtered through a Whatmann no 50 filter paper.
3. 1 ml of phosphotungstic acid was added to 5 ml of filtrate. This was mixed and centrifuged after 10 minute at 2000 rpm for 10 minutes and decanted. This precipitate was washed with 600 mmol/L of perchloric acid and again centrifuged.
4. The supernatant was drained.
5. 1 ml sodium carbonate solution, 3.5 ml water and 0.5 ml of phenol reagent was added and the tube was placed in water bath at 37ºC for 15 minutes.

6. Then absorbance was measured at 680 nm.
7. At the same time, a standard was put up of 0.5 ml tyrosine solution to which 1 ml sodium carbonate solution, 3 ml of water and 0.5 ml phenol reagent were added.
8. Both were read against a blank containing 3.5 ml water, 1 ml sodium carbonate and 0.5 ml phenol reagent.

Statistics

The data was analyzed by the students 't test and the ANOVA test. Pearson's coefficient was applied for correlational analysis.

Results

The aim of the study was to examine the level of α1- acid glycoprotein as an inflammatory marker in pathogenesis in diabetes mellitus. The mean age (range), body mass index (BMI) and values of random blood sugar (RBS) are presented in table 1. The control group participants were so chosen as to cover the age range of the test groups. Table 2 lists the values of α1- acid glycoprotein in all four groups as mean ± SD. Table 3 denoted the comparison between different groups and significance levels (p values). In newly diagnosed type 2 diabetic patients (Group II) show higher level of α1- acid glycoprotein in compare to control group (Group IV) as depicts the significant p value (< 0.0001*). It's also detect that the level of the α1- acid glycoprotein is significantly higher in newly diagnosed type 2 (Group II) in compare to newly diagnosed type 1(Group I) as denoted by significant p value (< 0.0001*). Even it is also shown that level of α1- acid glycoprotein is significantly declined after treatment by oral hypoglycemic drugs.

	Group I (n = 12) (Mean ± SD)	Group II (n = 25) (Mean ± SD)	Group III (n = 25) (Mean ± SD)	Group IV (n = 30) (Mean ± SD)
Age (yrs)	18.33 ± 7.64	48.22 ± 7.11	51.32 ± 7.56	44.97 ± 15.06
BMI	19.50 ± 1.23	24.03 ± 1.46	24.20 ± 2.40	21.75 ± 2.27
RBS	338.25 ± 50.97	193.26 ± 35.30	93.61 ± 33.65	94.20 ± 7.00

Table 1: *The anthropometric data of the subjects participated in the study are presented in Table 1.*

Serum Level (mg/dl)	Group I (Mean ± SD)	Group II (Mean ± SD)	Group III (Mean ± SD)	Group IV (Mean ± SD)
α1- acid glycoprotein (mg/dl)	94.87 ± 23.31	181.93 ± 31.94	87.10 ± 17.69	103.41 ± 22.13

Table 2: *The compare of mean value of α1- acid glycoprotein in groups in Table 2.*

Comparison Between Groups	Level (mg/dl)	Level (mg/dl)	p value
Comparison between Group I and Group IV	94.87 ± 23.31 (I)	103.41 ± 22.13 (IV)	0.275
Comparison between Group II and Group IV	181.93 ± 31.94 (II)	103.41 ± 22.13 (IV)	< 0.0001*
Comparison between Group III and Group IV	87.10 ± 17.69 (III)	103.41 ± 22.13 (IV)	0.005
Comparison between Group I and Group II	94.87 ± 23.31 (I)	181.93 ± 31.94 (II)	< 0.0001*
Comparison between Group II and Group III	181.93 ± 31.94 (II)	87.10 ± 17.69 (III)	< 0.0001*

Table 3: *Comparison of level of α1- acid glycoprotein (mg/dl) between different groups in Table-3 (p value < 0.05 is considered significant.)*

Group I = Type 1 diabetes mellitus patient (newly diagnosed)
Group II = Type 2 diabetes mellitus patient (newly diagnosed)
Group III = Type 2 diabetes mellitus patient (under treatment for at least 5 years)
Group IV = Control
n = number of subjects
SD = Standard Deviation
BMI= Body Mass Index
RBS= Random Blood Sugar
SD = Standard Deviation
*denoted significant value

Discussion

The aim of this study was to examine inflammation as a pathogenetic cause in type 1 and type 2 diabetes mellitus. In the twelve newly diagnosed type 1 patients, the level of α1-acid glycoprotein is not significantly elevated. In fact its seen lowering down (94.87 ± 23.31) in compare to control. (103.41 ± 22.13) and p value was not found as statistically significant (p 0.275). Previous reports of α1-acid glycoprotein in Type 1 diabetes were also contradictory. Crooke MA., *et al.* [14] has shown that serum α1-acid glycoprotein isnot elevated in type 1 diabetes mellitus but Gomes., *et al*. [15] reported increased level of CRP, α1-acid glycoprotein and fibrinogen in Type 1 patients.

Twenty-five type 2 newly diagnosed patients showed increased levels of α1-acid glycoprotein (Table-3) in compare to control as denoted by p value < 0.0001* which is considered as a statistically significant. The findings were in agreement with most of the authors who worked with α1-acid glycoprotein in type 2 diabetes [16,17]. The role of chronic low grade inflammation in the pathogenesis of type 2 diabetes seems possible beyond doubt. At the same time its role in type 1 diabetes cannot be completely ruled out. The course of the disease and resulting complications are similar in both type 1 and type 2 diabetes. The most dreaded complication being that of development of atherosclerosis resulting in cardiovascular diseases.

The values when compared between the untreated type 1 patient (Group I) and type 2 patients (Group II) reveals a significant increase in type 2 patients (Table-3). The mean random blood sugar (RBS) values in group 1(Type 1 diabetes) patients was 338.25 ± 50.97 mg/dl and that of group II (Type 2 newly diagnosed diabetics) was 193.26 ± 35.30 mg/dl. In spite of this huge difference, the inflammatory markers levels were higher in the type 2 patients which go to prove that the glycemic status doesn't influence the inflammatory markers (Table 1). This is in accordance with previous findings [18]. Evidence is available to say that inflammatory markers are elevated well before the clinical manifestation of hyperglycemia [19-22]. This also gives credence to the thought that activation of innate immunity is not related to hyperglycemia. But research has shown that decreasing plasma glucose levels decrease the concentration of acute phase reactants [23]. Also 2 hrs post load glucose values showed positive correlation with the inflammatory markers in few studies [18].

The underlying mechanism for the augmented acute phase response is not well understood and the stimulus for the response is unknown. A number of hypotheses have been put forward and these include insulin resistance, obesity, atherosclerosis, other diabetic complications and maladaptation of the normal innate immune response to environmental threats [24-26]. The most widely studied is the association of obesity, insulin resistance, type 2 diabetes and acute phase reactants. It has been shown that adipocytes secret a number of proinflammatory cytokines in the postprandial state [27-29]. The term 'diabecity' has received attention [30] of late for obese diabetics. The 'common soil' theory proposed, evaluates the involvement of inflammation in the pathogenesis of diabetes and atherosclerosis. Hyperglycemia and insulin resistance could promote inflammation and inflammation may be a factor linking diabetes mellitus to the development of atherosclerosis. Elevated glucose levels promote inflammation by increasing oxidative stress [31] by

increased TNF (kappa B) [32]. In this study, the mean BMI was found to be 19.5 ± 1.23 in type 1 patient and 24.03 ± 1.46 in type 2 patients. No correlation was found between BMI and acute phase reactants. Hence it can be summarized that there could be multiple pathways involved in the activation of the innate immunity system and much work needed to be done to establish either a casual role in the development of mainly type 2 diabetes and could be type 1 diabetes also.

Having demonstrated that there is an inflammatory process going on in type 2 diabetes, we next thought of estimating inflammatory markers in patients on treatment(for at least 5 years) with oral hypoglycemic drugs. Many of the drugs have been shown to have anti-inflammatory effects. Statin drugs inhibits HMG-CoA reductase and prevent atherosclerosis and inhibit the acute phase response by diminishing the deposition of LDL particles rich in cholesterol and phospholipids in macrophages and smooth muscle cells [33]. Statins were found to reduce CRP levels and did not correlate with the reduction of the lipid levels suggesting that the in addition to their ability to reduce LDL, statins may also inhibit the acute phase response .Freeman DJ et al showed that statin therapy also prevent diabetes mellitus [34]. The other very widely used oral hypoglycemic agents thiazolidinedions (Glitazone) are peroxizome proliferators activated receptor γ (PRAR γ) agonist that have been regarded as insulin sensitizes through mechanisms such as altered transcription of insulin sensitive genes controlling lipogenesis, adipocytes differentiation , fatty acid uptake and GLUT 4 (Glucose Transporter 4) expression. They also have an anti-inflammatory action inhibiting cytokine production, macrophage activation and reducing CRP as well as WBC count in type 2 diabetic subjects [35-38].

In this study, of the 25 type 2 diabetic patients on treatment for at least 5 yrs, 8 patient were in sulfonylurea-metformin combination, 7 were on glitazone, 6 were on sulfonylurea alone, 2 were on glitazone-metformin combination and 2 were on metformin alone. When compared with newly diagnosed untreated group (Group II) the levels of α1-acid glycoprotein was statistically lower (Table 3). The values of α1-acid glycoprotein was comparable to those of the control group. The RBS values were similar to those of untreated group (193.62 ± 33.65 and 193.26 ± 35.30). It is interesting to note that level of α1-acid glycoprotein is almost the same in type 1 diabetes, type 2 treated patients and control (Table 2). Probably α1-acid glycoprotein is the most amenable acute phase protein to treatment modalities in type 2 patient.

Conclusion and Limitation

For centuries we have known of the existence of two types of diabetes; the type 1, where the basic defect is an absolute deficiency of insulin due to an autoimmune destruction of the β cells and the type 2 diabetes, where the underlying pathology is decreased secretion of insulin or an increased resistance to the action of insulin by the insulin sensitive tissues. This simple classification was complicated by the emergence of a spectrum of overlapping patient characteristics and the disease proper. Newer sub classification like Maturity Onset Disease of the Young (MODY) which is type 2 diabetes in younger population and Latent Onset Autoimmune Diabetes of the Adults (LADA) which is type 1 diabetes in adults came in to existence. With this, the accepted pathogenesis also becomes questionable. Then come the era of finding newer and newer mechanisms involved it the pathology. One that received wide acceptance and paved way for further research is the role of activated innate immunity in the development of type 2 and probably type 1 diabetes. In continuation with the ongoing research world over we tried to examine whether this hypothesis holds true in a small subset of population. We can say with conviction that there is an activated innate immunity and a resultant increase in acute phase proteins in newly diagnosed type 2 diabetes as denoted by elevation of α1- acid glycoprotein which used to consider as a prominent inflammatory marker. But due to small sample size, we would say that the result should be interpreted with caution, although the inflammatory role cannot be ruled out. Irrespective of oral hypoglycemic drug used for the treatment, treated group showed significantly level of α1- acid glycoprotein comparable to the control group. What causes the innate immunity activation? Is it a cause or an effect of diabetes? What is the role of hyperglycemia? Is it associated with other complications of diabetes and if so, how? – are a few of the question which need to be addressed by intensive research. The mechanisms could be multifactorial and complex. A few hypothesis have been postulated which are still wanting.

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